VALIDATION RESULTS FOR METHOD NO. Covance Harrogate 2001-043-D1					
		MEAN	RELATIVE STD. DEV.(%)	RANGE	SEE (conc)
ANALYTE	Cotinine-N-ß-glucuronide				
MATRIX	Human urine				
DATE	19-Oct-01				
LLOQ	(Provide Statistical Criterion)	1 ng/mL. Accuracy 103.0%. RSD 11.4%			
STANDARD ERROR OF ESTIMATE OF QC SAMPLES (concentration units)					
(SEE = S.D./SQRT (# of determinations))					
	2 ng/mL	Low QC			0.069
	400 ng/mL	Med QC			3.899
	750 ng/mL	High QC			5.729
DESCRIPTIVE INFORMATION ON INSTRUMENT RESPONSE					
Function (Linear, Quadratic, etc.)	Linear				
Slope (mean)	0.0404				
Intercept (mean)	0.00658				
Other (mean regression value)	0.9983 ($1/x^2$ weighted)				
RECOVERY (as a %)					
High QC in (matrix)=	not assessed				
Med QC in (matrix)=	not assessed				
Low QC in (matrix)=	not assessed				
Results with different sample amounts (results of QC evaluations) intra-assay					
LLOQ QC=	1 ng/mL	1.03	11.4%	0.90-1.22	0.048
Low QC=	2 ng/mL	1.97	11.2%	1.82-2.33	0.090
Med QC=	400 ng/mL	359.60	2.1%	351.53-368.86	3.021
High QC=	750 ng/mL	685.29	1.8%	665.06-698.39	5.030
Dilution QC1=	2000 ng/mL	1882.90	1.8%	1834.9-1940.3	13.902
Dilution QC2=	20000 ng/mL	20342.24	2.7%	19737-21289	223.342
Results with different sample amounts (results of QC evaluations) inter-assay					
LLOQ QC=	n/a	n/a	n/a	n/a	n/a
Low QC=	2 ng/mL	1.93	14.7%	1.68-2.80	0.069
Med QC=	400 ng/mL	371.84	4.4%	350.93-397.57	3.899
High QC=	750 ng/mL	691.87	3.5%	652.91-755.16	5.729
STABILITY (assuming any degradation is linear):					
a) Room Temperature in Matrix (+/- %/24 hrs)		Stable			
b) Freeze/thaw (+/-% /3 cycles)		Stable			
c) Processed sample (+/- %/24 hrs)	not assessed	% maximum			

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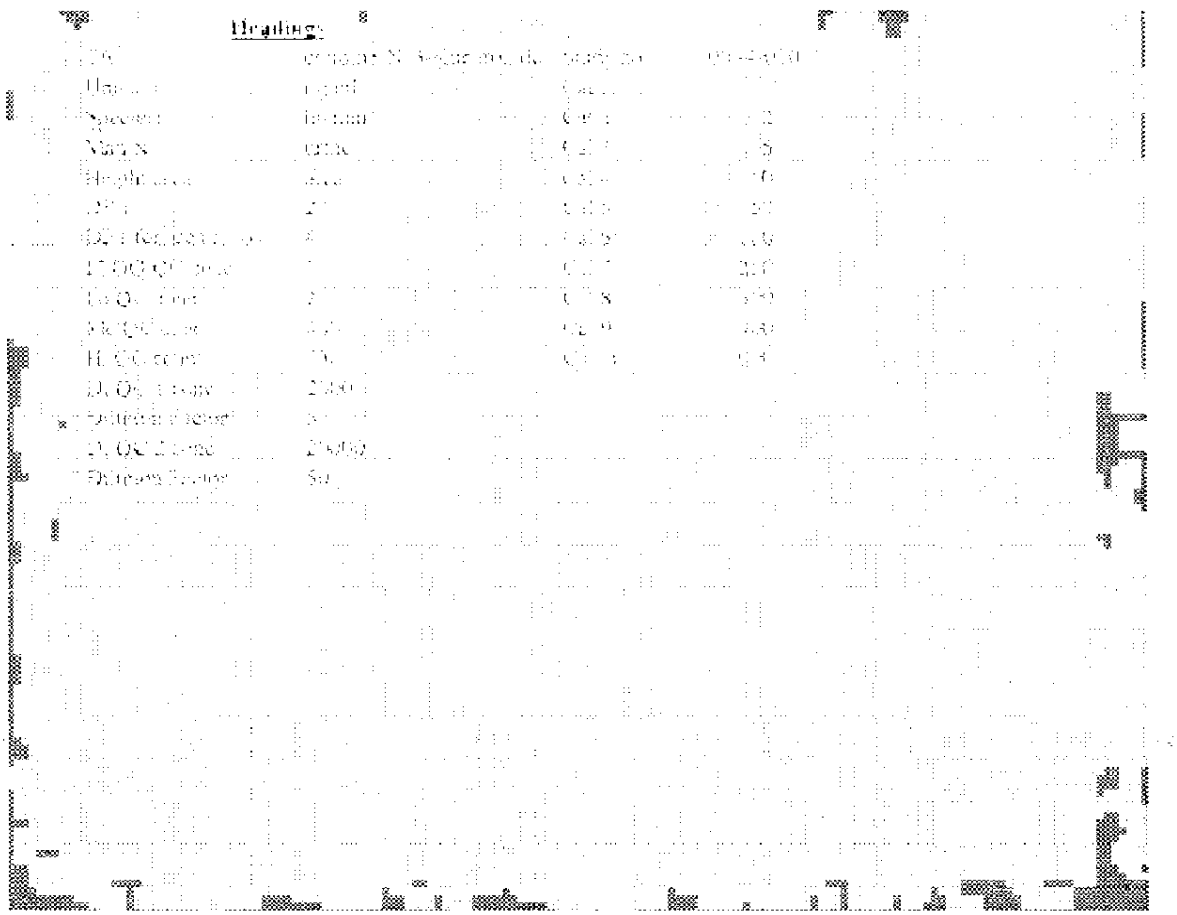

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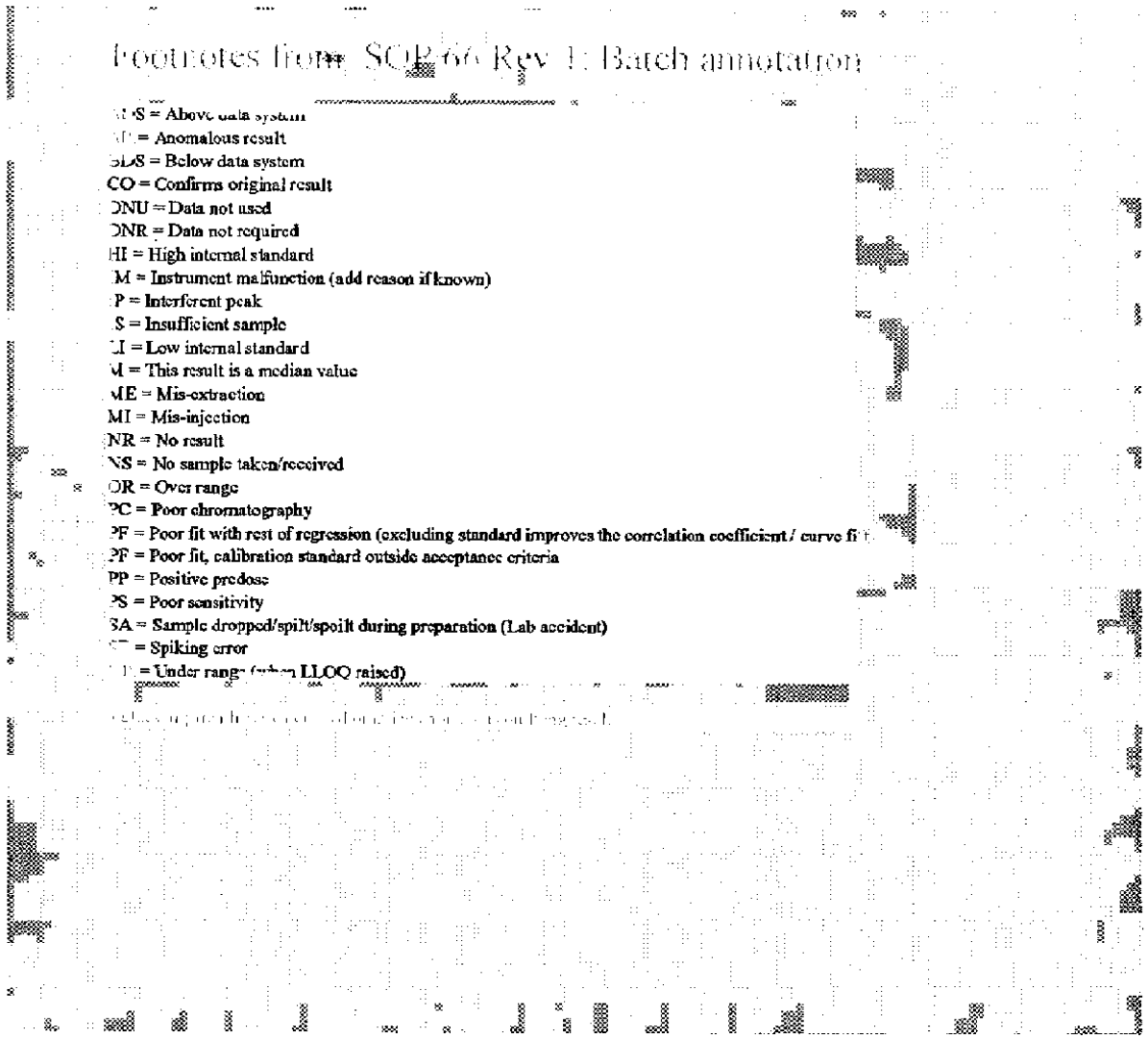

Footnotes from SOP 96 Rev 1: Batch annotation

AOS = Above data system
AR = Anomalous result
BLS = Below data system
CO = Confirms original result
DNU = Data not used
DNR = Data not required
HI = High internal standard
IM = Instrument malfunction (add reason if known)
IP = Interferent peak
IS = Insufficient sample
LI = Low internal standard
M = This result is a median value
ME = Mis-extraction
MI = Mis-injection
NR = No result
NS = No sample taken/received
OR = Over range
PC = Poor chromatography
PF = Poor fit with rest of regression (excluding standard improves the correlation coefficient / curve fit
PF = Poor fit, calibration standard outside acceptance criteria
PP = Positive predose
PS = Poor sensitivity
SA = Sample dropped/spilt/spoilt during preparation (Lab accident)
SE = Spiking error
UR = Under range (when LLOQ raised)

Source: https://www.industrydocuments.ucsf.edu/docs/lncj0001

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TABLE 1

Study no. 0044-020

Validation for the determination of cotinine-N-ß-glucuronide in human urine:
Intra-assay precision and accuracy data for cotinine-N-ß-glucuronide quality control samples

Replicate	QC 1 ng/mL (LLOQ QC)	QC 2 ng/mL (LoQC)	QC 400 ng/mL (MeQC)	QC 750 ng/mL (HiQC)	QC 2000 ng/mL (Di QC 1)*	QC 20000 ng/mL (DiQC 2)**
	Observed concentration (ng/mL)	Observed concentration (ng/mL)	Observed concentration (ng/mL)	Observed concentration (ng/mL)	Observed concentration (ng/mL)	Observed concentration (ng/mL)
1	0.93	1.82	365.99	697.79	1940.34	19933.02
2	1.00	2.08	368.86	680.69	1878.67	20163.11
3	0.90	1.73	353.16	685.37	1882.24	21288.52
4	1.02	1.83	363.48	698.39	1834.86	20512.80
5	1.22	2.03	351.53	665.06	1871.63	20419.13
6	1.10	2.33	354.55	684.43	1889.67	19736.88
Mean (ng/mL)	1.03	1.97	359.60	685.29	1882.90	20342.24
Standard deviation (n-1)	0.117	0.221	7.399	12.321	34.054	547.075
Precision (%)	11.4	11.2	2.1	1.8	1.8	2.7
Accuracy (%)	103.0	98.5	89.9	91.4	94.1	101.7

* analysed after 5-fold dilution with blank human urine
** analysed after 50-fold dilution with blank human urine

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TABLE 2

Study no. 0044-020

Validation for the determination of cotinine-N-β-glucuronide in human urine: Inter-assay precision and accuracy data for cotinine-N-β-glucuronide quality control samples

Batch	Replicate	QC 2 ng/mL (LoQC) Observed concentration (ng/mL)	QC 400 ng/mL (MeQC) Observed concentration (ng/mL)	QC 750 ng/mL (HiQC) Observed concentration (ng/mL)
HUVAL001RI	1	1.82	365.99	697.79
	2	2.08	368.86	680.69
	3	1.73	353.16	685.37
	4	1.83	363.48	698.39
	5	2.03	351.53	665.06
	6	2.33	354.55	684.43
HUVAL002RI	1	4.15 *	397.57	670.26
	2	1.69	371.53	755.16
	3	1.75	396.54	716.66
	4	1.99	388.57	705.34
	5	1.68	387.96	722.57
	6	1.75	390.13	696.51
HUVAL003	1	2.80	380.36	667.34
	2	1.87	350.93	686.60
	3	2.08	379.92	676.63
	4	1.83	351.63	652.91
	5	1.92	383.29	709.72
	6	1.69	357.14	682.26
Mean (ng/mL)		2.06 (1.93)	371.84	691.87
Standard deviation (n-1)		0.591 (0.284)	16.540	24.305
Precision (%)		28.7 (14.7)	4.4	3.5
Accuracy (%)		103.0 (96.5)	93.0	92.2
n		18 (17)	18	18

* Values in parentheses calculated omitting anomalous/outlying result

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TABLE 3

Study no. 0044-020

Validation for the determination of cotinine-N-ß-glucuronide in human urine:
Inter-assay precision and accuracy data for cotinine quality control samples

Batch	Replicate	QC 2 ng/mL (LoQC) Observed concentration (ng/mL)	QC 400 ng/mL (MeQC) Observed concentration (ng/mL)	QC 750 ng/mL (HiQC) Observed concentration (ng/mL)
HUVAL001RI	1	1.69	365.18	679.63
	2	2.30	364.89	673.43
HUVAL002RI	1	2.01	370.05	677.88
	2	1.62	389.51	707.06
HUVAL003	1	1.69	355.69	706.18
	2	1.91	341.16	628.58
Mean (ng/mL)		1.87	364.41	678.79
Standard deviation (n-1)		0.258	15.995	28.638
Precision (%)		13.8	4.4	4.2
Accuracy (%)		93.5	91.1	90.5
n		6	6	6

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TABLE 4

Study no. 0044-020

Validation for the determination of cotinine-N-β-glucuronide in human urine: Precision and accuracy data for cotinine quality control samples taken through the enzymatic deconjugation procedure

Batch	Replicate	QC 2 ng/mL (LoQC) Observed concentration (ng/mL)	QC 400 ng/mL (MeQC) Observed concentration (ng/mL)	QC 750 ng/mL (HiQC) Observed concentration (ng/mL)
HUVAL002RI	1	1.83	381.69	694.44
	2	1.77	378.64	725.58
	3	2.16	371.53	700.72
	4	1.70	374.39	708.96
	5	1.85	392.30	728.79
	6	1.91	382.98	721.69
Mean (ng/mL)		1.87	380.26	713.36
Standard deviation (n-1)		0.159	7.317	14.099
Precision (%)		8.5	1.9	2.0
Accuracy (%)		93.5	95.1	95.1
n		6	6	6

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TABLE 5

Study no. 0044-020

Validation for the determination of cotinine-N-ß-glucuronide in human urine: Precision and accuracy data for mixed (glucuronide/aglycone; 50:50 w/w) quality control samples taken through the enzymatic deconjugation procedure

Batch	Replicate	QC 2 ng/mL (LoQC)	QC 400 ng/mL (MeQC)	QC 750 ng/mL (HiQC)
		Observed concentration (ng/mL)	Observed concentration (ng/mL)	Observed concentration (ng/mL)
HUVAL002RI	1	1.91	389.67	686.74
	2	1.75	382.56	719.72
	3	1.87	378.60	667.78
	4	1.86	380.13	705.05
	5	1.86	374.88	687.94
	6	2.04	389.65	636.89
Mean (ng/mL)		1.88	382.58	684.02
Standard deviation (n-1)		0.094	6.023	29.067
Precision (%)		5.0	1.6	4.2
Accuracy (%)		94.0	95.6	91.2
n		6	6	6

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TABLE 6

Study no. 0044-020

Validation for the determination of cotinine-N-ß-glucuronide in human urine:
Inter-assay precision and accuracy data for calibration standards

Batch	Back-calculated concentrations (ng/mL)										Curve parameters		
	Calibration level (ng/mL)										Gradient (m)	Intercept (c)	Correlation coefficient (r) *
	1	2	5	10	50	100	250	500	900	1000			
IIUVAL001RI	0.95	2.21	4.82	10.24	47.82	97.65	258.95	506.49	894.67	977.63	0.0418	0.00382	0.9987
IIUVAL002RI	0.97	2.25	4.49	9.28	50.31	101.66	238.58	541.94	932.33	988.97	0.0391	0.00756	0.9971
IIUVAL003	1.00	2.07	4.75	9.59	48.78	95.76	263.16	510.04	927.19	1023.27	0.0403	0.00837	0.9992
Mean (ng/mL)	0.97	2.18	4.69	9.70	48.97	98.36	253.56	519.49	918.06	996.62	-	-	-
Standard deviation (n-1)	0.025	0.095	0.174	0.490	1.256	3.013	13.146	19.523	20.422	23.763	-	-	-
Precision (%)	2.6	4.4	3.7	5.1	2.6	3.1	5.2	3.8	2.2	2.4	-	-	-
Accuracy (%)	97.0	109.0	93.8	97.0	97.9	98.4	101.4	103.9	102.0	99.7	-	-	-

* the coefficient of determination (r^2) is the square of the correlation coefficient

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TABLE 7

Study no. 0044-020

Validation for the determination of cotinine-N-ß-glucuronide in human urine:
Room temperature stability of cotinine-N-ß-glucuronide in human urine

Quality control level (ng/mL)		Replicate Deconjugated QC samples		Baseline QC samples (no deconjugation)		QC samples stored for 24 h prior to extraction (no deconjugation)	
		Observed Peak Area Ratio	Mean	Observed Peak Area Ratio	Mean (Aglycone %)	Observed Peak Area Ratio	Mean (Aglycone %)
2	1	0.1211	0.0902	0.0091	0.0091	0.0090	0.0076
	2	0.0835		0.0117	(10.1)	0.0079	(8.4)
	3	0.0922		0.0069		0.0060	
	4	0.0822		0.0061		0.0071	
	5	0.0858		0.0133		0.0083	
	6	0.0764		0.0074		0.0074	
750	1	26.8784	27.3577	0.0232	0.0209	0.0233	0.0229
	2	27.6539		0.0180	(0.1)	0.0237	(0.1)
	3	27.2523		0.0188		0.0208	
	4	26.2972		0.0229		0.0248	
	5	28.5849		0.0206		0.0221	
	6	27.4792		0.0218		0.0229	

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TABLE 8

Study no. 0044-020

Validation for the determination of cotinine-N-ß-glucuronide in human urine:
Freeze/thaw stability of cotinine-N-ß-glucuronide in human urine

Quality control level (ng/mL)		Replicate Deconjugated QC samples		Baseline QC samples (no deconjugation)		QC samples subjected to 3 additional freeze/thaw cycles prior	
		Observed Peak Area Ratio	Mean	Observed Peak Area Ratio	Mean (Aglycone %)	Observed Peak Area Ratio	Mean (Aglycone %)
2	1	0.1211	0.0902	0.0091	0.0091	0.0082	0.0071
	2	0.0835		0.0117	(10.1)	0.0079	(7.9)
	3	0.0922		0.0069		0.0047	
	4	0.0822		0.0061		0.0055	
	5	0.0858		0.0133		0.0092	
	6	0.0764		0.0074		0.0073	
750	1	26.8784	27.3577	0.0232	0.0209	0.0257	0.0205
	2	27.6539		0.0180	(0.1)	0.0224	(0.1)
	3	27.2523		0.0188		0.0166	
	4	26.2972		0.0229		0.0209	
	5	28.5849		0.0206		0.0176	
	6	27.4792		0.0218		0.0196	

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TABLE 9

Study no. 0044-020

Validation for the determination of cotinine-N-ß-glucuronide in urine area:
Stability of stock solutions stored at nominal 4°C for X days

Date of stock solution preparation	Replicate	Peak area of stock solution.	Mean peak area value	% Difference of stored solution from new solution
xx/xx/xx (new)	1		#DIV/0!	#DIV/0!
	2			
	3			
	4			
	5			
	6			
xx/xx/xx (stored)	1		#DIV/0!	
	2			
	3			
	4			
	5			
	6			

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Source: https://www.industrydocuments.ucsf.edu/docs/lncj0001